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Etiological stratification and clinical implications of indirect combs test positivity in Rh-incompatible pregnancies: A retrospective cohort analysis from a tertiary care center

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Abstract

The Indirect Coombs (IDC) is a screening test used to detect maternal sensitization in pregnancies with Rh incompatibility. This study aims to investigate the prevalence and underlying causes of IDC test positivity in our clinic, as well as to evaluate its associated prenatal and postnatal outcomes. The study was conducted at Health Sciences University, Istanbul Training and Research Hospital, Department of Obstetrics and Gynecology, between August 2016 and 2019. The records of Rh D-negative pregnant women aged 18-40, who underwent Indirect Coombs testing for Rh incompatibility screening, were retrospectively reviewed in the system. Pregnancies with positive IDC test results were included in the study and categorized into three main groups. Group I consisted of cases with Non-Anti-D-mediated IDC positivity. Group II included cases with IDC positivity induced by Anti-D immunoglobulin prophylaxis. Group III consisted of patients with Anti-D-mediated IDC positivity without a history of immunoglobulin prophylaxis. The prenatal and postnatal outcomes of these groups were evaluated and compared. A total of 185 positive IDC test results were identified. Group I, Group II, and Group III comprised 63 (34%), 95 (51.4%), and 27 (14.6%) patients, respectively. In both Group I and Group II, even in patients with high IDC test titers, no cases of prenatal or postnatal fetal anemia were observed. In contrast, Group III demonstrated significantly higher rates of the following parameters compared to Groups I and II: evidence of fetal impact on ultrasound examination, fetal hydrops, anemia in the newborn, neonatal ICU admission, need for exchange transfusion and IVIG therapy, jaundice, phototherapy requirement, and newborn Direct Coombs positivity (z-test, $p < 0.05$). No significant differences were found between Groups I and II regarding these parameters, and fetal outcomes were similar in these groups.In our study, most of the positive results were due to non-Anti-D-mediated causes and previous Anti-D immunoglobulin prophylaxis applications, with similar prenatal and postnatal prognoses in these two groups. However, fetal and neonatal morbidity and mortality rates were significantly higher in Group III: the "affected Rh-D disease" group.

Keywords: Anti D immunoglobulin prophylaxis, Hemolytic disease of fetus and newborn, Indirect coombs test, Rh incompatibility

Introduction

Rh incompatibility is primarily influenced by the prevalence of Rh D-negative blood types, which varies significantly among populations. This condition arises when a Rh D-negative pregnant woman carries a fetus with Rh D-positive blood type, typically inherited from the father. If fetal Rh D-positive erythrocytes cross into the maternal circulation—most commonly during delivery, but potentially earlier due to events such as miscarriage, vaginal bleeding, chorionic villus sampling, or pregnancy termination the maternal immune system may recognize the D antigen as foreign and initiate an immune response.

This initial exposure results in maternal sensitization; wherein anti-D antibodies are produced. Once formed, these antibodies can traverse the placenta in subsequent pregnancies and target the red blood cells of a Rh D-positive fetus, leading to hemolysis. The severity of this immune-mediated destruction can range from mild hemolytic disease of the newborn (HDN) to severe outcomes such as hydrops fetalis, high-output cardiac failure, or intrauterine fetal demise (1).

Despite the widespread availability of effective prophylactic measures, including anti-D immunoglobulin administration, Rh alloimmunization remains a preventable yet significant public health concern in low-resource settings. It is estimated that approximately 0.3% of susceptible women continue to develop Rh D alloimmunization, underscoring the need for improved screening and preventive strategies in these populations (2, 3).

The Indirect Coombs (IDC) test detects anti-D antibodies in the maternal serum. It is strongly

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recommended that Rh-D blood type and antibody screening be performed for pregnant women during the initial prenatal visit. Early detection allows for timely intervention to prevent or reduce the severity of hemolytic disease of newborn (1). Additionally, it is advised to repeat IDC testing for all unsensitized Rh-D negative mothers between 24 and 28 weeks of gestation, unless the father is confirmed to be Rh-D negative. Antibody testing should also be conducted at the time of delivery (4).

Routine antenatal anti-D prophylaxis, introduced at the beginning of the third trimester in several countries including Türkiye, has effectively reduced the incidence of Rh-D immunization (5).

Anti-D prophylaxis involves the use of pooled polyclonal anti-D IgG derived from human plasma donors. Routine antenatal anti-D prophylaxis (RAADP) should be administered to all Rh-D negative pregnant women at 28 weeks of gestation who are at risk for Rh incompatibility (6).

If the neonate is confirmed to be Rh-D positive after delivery, the same unsensitized Rh-D negative women should receive Rh-immunoglobulin (RhIg) within 72 hours postpartum. In Türkiye, the recommended RhIg dose is usually 300 mcg, which is sufficient to neutralize up to 15 mL of Rh-D positive erythrocytes (equivalent to 30 mL of whole fetal blood) (4).

Once a woman becomes Rh-D immunized, all her subsequent pregnancies with a Rh-D–positive fetus are uner the risk of being affected (6).

In sensitized pregnancies, in which alloimmunization has already occurred, no further administration of anti-D is required. Positive IDC test necessitates titration to assess the antibody concentration. Serial antibody titers are typically measured every four weeks until 28 weeks of gestation and every two weeks thereafter. Traditionally, when titers reach a laboratory-specific 'critical' level - commonly defined as ≥1:16 for anti-Rh-D - it usually indicates a higher risk of fetal anemia positively correlated with increasing titers (7).

In order to achieve secondary prevention of hemolytic disease of the fetus and newborn (HDFN), of Anti-D immunoglobulin," and Group III (n=27): these patients should be referred to a maternal-fetal medicine specialist for pregnancy surveillance. This includes serial ultrasound scans and monitoring for the development of fetal anemia using middle cerebral artery (MCA) Doppler velocities (8). It cannot be initiated before 18 weeks of gestation and due to the likelihood of a false-positive result; it requires caution after 35 weeks (3).

The aim of this study is to investigate the causes of IDC positivity among Rh-D negative pregnant women who were screened for Rh incompatibility and their varying impact on fetal-neonatal well-being.

Methods

The study was conducted at Health Sciences University, Istanbul Training and Research Hospital, Department of Obstetrics and Gynecology, between August 2016 and 2019. Approval was granted by the local Ethics Committee of Istanbul Training and Research Hospital. (date: 13.09.2019, decision number: 1977).

Out of 12,173 pregnant women, the data of 213 Rh-D negative pregnant women aged 18–40 years with positive IDC results were retrospectively analyzed using a computerized storage system. Among these, 185 women with available follow-up and delivery information were included in the study, and duplicate test results were excluded from the analysis. At the laboratory, hemagglutination screening and titration were performed using the Across System® Automatic System Octo-m device with automatic gel centrifugation method, utilizing Across LISS solution and Across Gel cards for the indirect Coombs test procedure. In our center, positive IDC test results were reported as positivity either “caused by Anti-D antibodies” or “not caused by Anti-D” regardless of titer. IDC test positivities for the cases detailed as “caused by Anti-D” were considered to be candidates for Rh disease. The medical histories of these patients were examined meticulously, and those with a recent history of immunoglobulin prophylaxis before the IDC test, were grouped as IDC-positive cases attributed to the administered Anti-D immunoglobulin Thus, cases with positive IDC tests were divided into three main groups based on their causes: Group I (n=63): "Non-Anti-D related cases," Group II (n=95): "Cases related to the administration "Cases considered as possible Rh disease”.

Demographic characteristics of the patients such as maternal age, gravida, parity, number of abortions/curettages, living children, and maternal blood types were recorded. Clinical data included IDC test titers, fetal ultrasound findings, need for intrauterine transfusion, and pregnancy outcomes (live birth, IU fetal demise, abortion). Neonatal outcomes such as birth weight, jaundice, presence of anemia, need for intensive care, phototherapy, exchange transfusion and intravenous immunoglobulin (IVIG), Direct Coombs (DC) positivity, and neonatal mortality were noted through hospital records and patient interviews. The greatest of effort was exerted to obtain as much objective and trustworthy data as possible and comparisons were made between groups regarding these outcomes. Available perinatologic evaluation and data were highly standardized since all were obtained by the same expert using the same ultrasonography device (Samsung Electronics Co., HS70A).

Sonographic evaluation for fetal anemia included assessment of middle cerebral artery peak systolic velocity (MCA-PSV), with values >1.5 MoM considered suggestive of moderate to severe anemia, as well as structural markers such as hydrops fetalis (ascites, skin edema, pericardial/pleural effusion), cardiomegaly, and placental thickening (9, 10). Neonatal anemia was defined as a hemoglobin level below 13.5 g/dL, Hematocrit < 40% at birth, consistent with thresholds established in standard neonatal references and previous literature (11). Fetal hematocrit value used as the threshold for requiring a post-partum exchange transfusion was 30% (12).

Fetal hydrops is defined as the presence of 2 abnormal fluid collections in the fetus. These include ascites, pleural effusions, pericardial effusion, and generalized skin edema (defined as skin thickness >5mm) (13).

Statistical analysis

All data were recorded and analyzed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics, including mean, standard deviation, median, frequency, percentage, minimum, and maximum, were used to summarize the data. Frequency analyses were conducted, and means and standard deviations were calculated where appropriate.

To assess differences between groups, one-way ANOVA was used for normally distributed continuous variables, while the Kruskal–Walli's test was applied for non-normally distributed continuous variables. Post hoc comparisons following ANOVA were performed using Tukey's Honestly Significant Difference (HSD) test. For categorical variables, the Chi-square test or Fisher's exact test was used depending on the distribution and sample size. Pairwise comparisons of categorical variables were performed using Z tests with Bonferroni correction. A p value < 0.05 was considered statistically significant for overall group comparisons.

Results

Upon analysis of 213 cases with detected IDC positivity (1.73%), the number of Non-Anti-D related cases was 63 (29.6%) (Group I), and the number of cases caused by Anti-D antibodies was 150 (70.4%). Out of these 150 cases, complete pregnancy follow-up records were accessible for 122, all of which were included in the study. Among these, 95 (77.9%) were classified as Group II (related to Anti-D administration), and 27 (22.1%) as Group III (related to possible Rh disease). A total of 185 pregnant women were analyzed.

The average maternal age of women was 28.9 years. In terms of demographic characteristics, no statistically significant difference was observed between the groups for maternal age and number of abortions ($p > 0.05$). Gravida, parity, and the number of living children were found to be significantly higher in Group III compared to other two groups, p value < 0.05. (Table 1).

Table 1. Characteristics of pregnant women based on groups defined by indirect coombs positivity and anti-D alloimmunization status

	Group 1	Group 2	Group 3	P
N	63	95	27	
Age years	29.7±5.1	29.6±5.4	31.8±5.9	0.137
Gravida	2.7±1.5	2.1±1	4.3±2	0.000
Parity	1.4±1.2	0.8±0.8	2.5±1.6	0.000
Abortus	0.3±0.3	0.3±0.3	0.7±1.1	0.251
Living	1.2±1.1	0.8±0.9	2.3±1.4	0.000

Data represented as mean± standard deviation, p value < 0.05

With the best of available data depicted in Table 2, gestational week at delivery and birth weight of the neonate were significantly lower in Group III, although there was no difference in between Group I and II. However, no significant difference was observed between groups in terms of the percentage of cesarean deliveries (p > 0.05).

Neonatal anemia was not observed in Groups 1 and 2, but was significantly more frequent in Group 3 (45.8%, $p < 0.001$). Similarly, NICU admissions were markedly higher in Group 3 (87.5%) compared to Group 1 (33.3%) and Group 2 (37.5%) ($p < 0.001$). Postpartum transfusions were required exclusively in Group 3 (33.3%), with no such interventions needed in other groups ($p < 0.001$). Although the incidence of neonatal death did not differ significantly among groups ($p = 0.257$), jaundice and the need for phototherapy were significantly more prevalent in Group 3 (95.8% and 83.3%, respectively) compared to Groups 1 and 2 ($p < 0.001$ for both). Intravenous immunoglobulin (IVIG) therapy was administered only in Group 3 (37.5%; $p < 0.001$). Furthermore, direct Coombs (DC) test positivity in neonates was significantly higher in Group 3 (79.2%) compared to the other groups ($p < 0.001$). These findings underscore the association between maternal alloimmunization and adverse neonatal hematologic outcomes, particularly in Group 3 patients who exhibited serologic evidence of anti-D-mediated hemolysis.

Group 3 showed higher rates of neonatal anemia, NICU admission, postpartum transfusion, jaundice, phototherapy, IVIG administration, and neonatal direct Coombs positivity compared to Groups 1 and 2 ($p < 0.001$ for all). (Table 2)

Table 2. Neonatal Outcomes Based on Groups Defined by Indirect Coombs Positivity and Anti-D All immunization Status

	Group 1	**Group 2**	**Group 3**	**P**
N	24	40	24	
Gestational age at birth	38±2.9	38.6±1.6	35.6±5.5	0.005*
Birthweight	3177±677	3396±448	2829±978	0.007*
Cesarean section rate (%)	41.7	52.5	59.3	0.450
Neonatal anemia	0, (0)	0, (0)	11, (45.8)	0.000*
NICU admission	8, (33.3)	15, (37.5)	21, (87.5)	0.000*
Postpartum transfusion	0, (0)	0, (0)	8, (33.3)	0.000*
Neonatal death	0, (0)	1, (2.5)	2, (8.3)	0.257
Jaundice	11, (45.8)	18, (45)	23, (95.8)	0.000*
Phototherapy	8, (33.3)	11, (27.5)	20, (83.3)	0.000*
IVIG	0, (0)	0, (0)	9, (37.5)	0.000*
Neonatal DC positivity	1, (4.2)	1, (2.5)	19, (79.2)	0.000*

N: live births Data represented as N, (%) *p value < 0.05

In Group I (n=63), of the 29 cases with known pregnancy outcomes, 4 (13.8%) resulted in abortion, and 1 (3.4%) with positive auto-immune antibodies (anti-nuclear and anti-thyroid peroxidase; ANA and Anti-TPO) resulted in intrauterine fetal demise. The total number of pregnancy losses for this group was 5 (17.2%), while 24 cases (82.8%) resulted in live births. None of these 24 cases showed signs of fetal involvement, fetal hydrops, presence/development of fetal anemia, need for intrauterine transfusion, postpartum exchange transfusion, IVIG, or ended in postpartum neonatal death.

In this group, only 4 cases had available MCA-PSV measurement results, all of which were normal. Two of these cases were notable. In one, MCA Doppler was performed due to a surprisingly high IDC titer of 1/256, and in the other there was a history of maternal rash-related illness.

Among 24 patients with live births, postpartum jaundice was present in 11 cases (45.8%), while 8 cases (33.3%) required neonatal intensive care (NIC) and phototherapy.

For Group I, majority of IDC positivity was not at a titer-reporting level with the exception of 3 cases (4.8%) with titers of 1/2, 1/16 and the aforementioned 1/256. In this group only 1 case (4.2%) with the titer 1/256 also had a post-partum positive DC result. Despite this, the newborn did not present with anemia at birth, and prenatal Doppler assessment of MCA-PSV, yielded normal results. This was attributed to potential subgroup incompatibilities not documented in the patient's medical history.

In our study, 7 patients (11.1%) in Group I (n=63) exhibited identifiable non-anti-D-related causes for IDC positivity. Among these, one patient tested positive for Rubella IgM, and another had a maternal rash illness. Three patients had a documented history of medication use: Methyldopa (Alfamet®) in two cases and Duloxetine (Cymbalta®) in one. Furthermore, two patients had maternal autoimmune conditions—one diagnosed with Behçet's disease and ankylosing spondylitis, and another with systemic lupus erythematosus concomitant with thyroid disease, exhibiting antinuclear antibody (ANA) and anti-thyroid peroxidase (Anti-TPO) antibody positivity.

In Group II, pregnancy outcomes of 46, out of 95 patients were known. Among these 46 patients, 6 (13.0%) pregnancies resulted in abortion, while 40 (87.0%) resulted in live births. In this group, there were no signs of fetal anemia or findings suggestive of fetal hydrops. None of the cases required intrauterine / exchange transfusion, or IVIG. Out of 40, postpartum jaundice was observed in 18 (45.0%), phototherapy was required in 11 (27.5%), and NIC was needed in 15 cases (37.5%). 1 newborn (2.5%) was lost in postpartum period due to respiratory distress.

Among 95 cases of Group II, IDC positivity titers were measured in 32. The recorded maximum level was 1/32, and the median titer value was 1/4. Only 1 case (2.5%) had a positive DC result.

In Group III, 24 (88.9%) out of all 27 pregnancies with available prenatal follow-up information, resulted in live births, while 3 (11.1%) ended in fetal loss, including 1 intrauterine demise (3.7%) and 2 pregnancy terminations (7.4%). The case with intrauterine fetal demise was the one with the highest IDC titer positivity (1/2048) and experienced demise at 20 weeks due to hydrops. Both pregnancies that were terminated had IDC titers of 1/256; one at 22 weeks due to early membrane rupture, and the other at 20 weeks due to early hydrops and cardiomegaly. Of 24 live-born babies, 2 (8.3%) died in neonatal period whose maternal IDC titers were 1/128. Among 27, 8 cases (29.6%) showed signs of fetal involvement, 4 of which (14.8%) presenting with hydrops. No postpartum survival was achieved in cases of fetal hydrops. For the other non-hydropic 4 cases, fetal anemia was detected by MCA Doppler velocimetry. Of these 4, 3 cases with accessible postnatal follow-up resulted in positive survival outcomes. Only 1 of these 3, with an IDC positivity titer of 1/1024, received appropriate and timely perinatology counseling/care and underwent intrauterine transfusion. The baby was delivered at 31 weeks due to bradycardia developing during intrauterine intervention. The other two with IDC titers of 1/256 and 1/512 were delivered at 35th and 34th gestational weeks, respectively without the need for intrauterine transfusion. All three neonates required NIC follow-up, postpartum transfusions, and treatment for neonatal jaundice but were discharged in good health after successful treatment. The follow-up and delivery information for the 4th case with detected anemia and an IDC titer of 1/32 could not be obtained.

Among 24 cases that resulted in live births, neonatal anemia was observed in 11 (45.8%) and jaundice in 23 (95.8%). Exchange transfusion was required in 8 cases (33.3%), IVIG administration in 9 (37.5%), phototherapy in 20 (83.3%), and NIC follow-up in 21 (87.5%). These values were significantly higher than those of the first two groups. In this group, the highest recorded titer level was 1/2048, with a median titer value of 1/256.

Among the 24 live-born babies, 21 (87.5%) were Rh-D positive, and 3 (12.5%) were Rh-D negative. The IDC titers of the mothers of fetuses with Rh-D negative blood groups were relatively low during pregnancy follow-ups (1/8, 1/8, 1/64) and showed a decreasing trend throughout the pregnancy (1/4, 1/2, 1/16, respectively).

In this group, 19 cases (79.2%) had a positive DC result. Of the 5 babies with negative DC results, one was of an anemic baby with a Rh-D (+) blood group

which was interpreted as a false negative. Another Rh-D (+) baby without anemia had a relatively low maternal IDC titer (1/8). All the remaining 3 babies with negative DC results had a Rh-D (-) blood group.

There was no case of spontaneous abortion in this group. However, with the exception of the 22nd week early membrane rupture termination, the other 4 losses among the total 5 (80%), can be directly attributable to Rh disease.

When three groups of the study (Group I, Group II, Group III) were compared in terms of fetal involvement findings during pregnancy follow-up no statistically significant differences were found between Group I and Group II for these parameters. However, when the–latter 2 were compared with Group III, adverse perinatal and neonatal indicators were more frequent in Group III than in Group I and Group II. The difference was deemed significant based on z-test result ($p < 0.05$).

Sonographic abnormalities (29.6%), fetal hydrops (14.8%), and fetal anemia (14.8%) were observed exclusively in Group 3, with statistically significant differences compared to Groups 1 and 2 ($p < 0.01$ for each). While intrauterine transfusion and fetal loss occurred only in Group 3, these differences did not reach statistical significance. Spontaneous abortion was observed in Groups 1 and 2, it was not detected in Group 3 ($p<0.05$). However, there was no statistically significant difference in abortion rates between Group 1 and Group 2. (Table 3)

Table 3. Fetal findings based on groups defined by indirect coombs positivity and anti-D alloimmunization status

	Group 1	Group 2	Group 3	p
N	29	46	27	
Sonographic findings	0 (0.0%)	0 (0.0%)	8 (29.6%)	<0.001*
Fetal hydrops	0 (0.0%)	0 (0.0%)	4 (14.8%)	0.006*
Fetal anemia	0 (0.0%)	0 (0.0%)	4 (14.8%)	0.006*
Intrauterine transfusion	0 (0.0%)	0 (0.0%)	1 (3.7%)	0.298†
Fetal loss	1 (3.4%)	0 (0.0%)	3 (11.1%)	0.101†
Spontaneous abortion	4 (13.8%)	6 (13.0%)	0 (0.0%)	0.048*

*: Sonographic findings, fetal anemia, and fetal hydrops were observed exclusively in Group 3, with statistically significant differences. ($p < 0.05$)

*: Spontaneous abortion was reported in Groups 1 and 2 but was absent in Group 3, which was also statistically significant. ($p < 0.05$)

†: There were no significant differences among groups for intrauterine transfusion and fetal loss.

Among patients in Group 3, the correlation between the highest recorded IDC titer values and presence of neonatal anemia was scrutinized. Data regarding titer levels and neonatal anemia status both were available for 21 patients. Upon further evaluation, IDC titers—particularly those above 1:64—were depicted to be more frequently associated with neonatal anemia. (Figure 1).

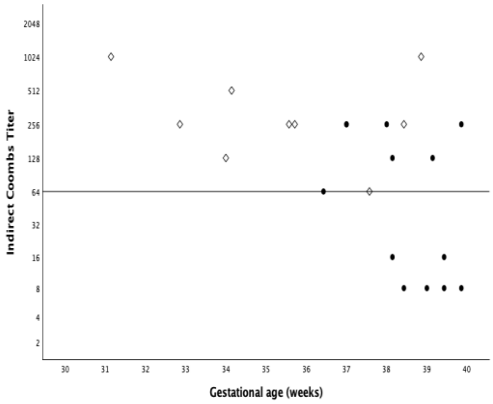

Figure 1. Highest indirect Coombs titer values of fetuses in Group 3 based on neonatal anemia status at birth

The scatter plot displays indirect Coombs test (IDC) titers across gestational age at delivery, horizontal line marks the titer threshold of 1:64, commonly considered clinically significant.

"•" represents infants without neonatal anemia at birth

"◊" represents infants with neonatal anemia at birth

When examining the relationship between the highest recorded IDC titer values and sonographic evidence of fetal anemia, data was available for 23 cases. (Figure 2) The findings suggest that elevated IDC titers, particularly those exceeding 1:128, are more frequently associated with sonographic indicators of fetal anemia, underscoring a potential correlation between higher maternal antibody levels and in-utero hematologic compromise.

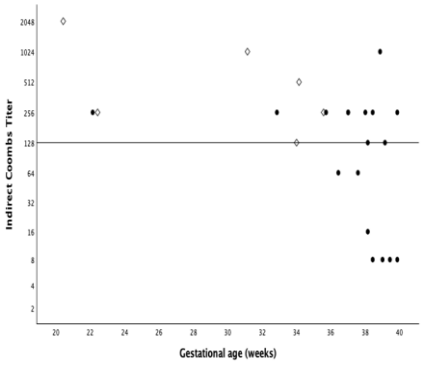

Figure 2. Highest indirect Coombs titer values of fetuses in Group 3 based on sonographic evidence of anemia during pregnancy

The scatter plot illustrates the distribution of indirect Coombs test (IDC) titers in relation to gestational age

"·" represents fetuses without sonographic evidence of anemia

"◊" represents fetuses with sonographic evidence of anemia

Discussion

Rh D disease is a foreseeable condition which, with screening, allows for early diagnosis and with timely interventions, leads to a favorable pre- and postnatal prognosis (14). Despite the widespread use of anti-D immunoglobulin prophylaxis worldwide, the prevalence of Rh disease is still estimated to be 276 per 100,000 live births (0.28%) (4). In our clinic, Rh disease was detected in 27 cases (0.22%) out of 12,173 births, a rate comparable to the prevalence reported in the literature.

Although numerous studies have investigated the prevalence of Rh-D alloimmunization and its neonatal consequences, (15) few have specifically examined the etiological distribution of IDC test positivity by differentiating anti-D from non–anti-D antibodies, or assessed the proportion of anti-D-related positivity attributable to true maternal alloimmunization as opposed to passive sensitization due to prophylactic administration of Rh immunoglobulin. Our study aims to contribute to the literature by quantifying the rate of IDC test positivity observed during routine prenatal screening among Rh-incompatible couples, classifying the underlying causes of this positivity with respect to Rh-D immunization status, and delineating the proportion of fetuses affected by true Rh hemolytic disease. Our findings align with existing literature reporting an incidence of indirect Coombs test (IDC) positivity in 1% to 4% of pregnancies (16,17), with a substantial majority attributed to anti-D antibodies, typically ranging from 60% to 70% (18). In our cohort of 213 IDC-positive cases (1.74% of screened pregnancies), 70.4% were associated with anti-D antibodies, reinforcing these previously reported proportions. Notably, etiologic disaggregation revealed that among the 122 anti-D related cases with complete follow-up data, a significant proportion (77.9%) stemmed from passive anti-D immunoglobulin administration (Group II), whereas only 22.1% were linked to probable Rh alloimmunization (Group III). This distribution corroborates prior evidence suggesting that with the widespread adoption of Rh prophylaxis, true sensitization events represent a minor subset—estimated at 0.1%–0.4% of pregnancies (19,20). Our data underscore the critical importance of distinguishing passive from active alloimmunization to avoid unnecessary interventions and anxiety, particularly in routine prenatal care. The observed rates validate the clinical relevance of etiologic stratification in IDC positivity, advocating for more nuanced interpretation strategies in perinatal immunohematology.

Upon examining causes of IDC positivity other than anti-D antibodies and antigen subgroup incompatibilities, it has been observed that certain conditions leading to Direct Coombs positivity can also result in IDC test positivity. Examples include active Rubella infection (21) and Hepatitis E (22), both of which can cause autoimmune hemolytic anemia and a positive direct Coombs test.

As is well known, drug-induced hemolytic anemia can occasionally result in simultaneous positivity of both direct and indirect Coombs tests. Snyder et al.

presented a case of hemolytic anemia associated with Methyldopa (23), while Örüm reported a case linked to selective serotonin reuptake inhibitor use (24), both of which exhibited positivity in both Coombs tests.

Subgroup antigen incompatibilities—particularly Kell, Duffy, and Kidd—are also implicated in HDFN. Kell incompatibility is the most severe, often suppressing fetal erythropoiesis, whereas Duffy typically causes moderate hemolysis, and Kidd leads to mild, delayed jaundice (25). ACOG notes that while Kell alloimmunization is rare, it can result in significant fetal anemia, with maternal titers occasionally reaching 1:128 (26). In such cases, laboratory identification of subgroup antigens is ideal. However, where unavailable, high-titer results should prompt vigilant fetal anemia screening via middle cerebral artery Doppler studies (27). The inability to assess subgroup antigens in our study represents a key limitation.

Following the administration of Anti-D immunoglobulin, antibody titers are generally not expected to exceed 1:2 (28). Behrens et al. reported maximum post-prophylaxis titers of 1:8 (29), while Andres et al. described a case reaching 1:16 following a 300-mcg dose (28). In the present study, among patients in Group II, the maximum recorded titer was 1:32, with a median titer of 1:4. Various factors may influence antibody titers, among which, the timing of post-vaccination blood sampling appears to be particularly significant. However, in cases where Rh sensitization due to underlying alloimmunization is present, vaccine-induced antibody positivity may misguide interpretation.

Analysis of overall pregnancy outcomes in Group II demonstrated that IDC positivity attributable to Anti-D immunoglobulin administration did not adversely affect fetal prognosis. This finding underscores the importance of obtaining a thorough history regarding the administration and timing of Anti-D immunoglobulin. It is essential to recognize that positive IDC results obtained after prophylactic Anti-D administration may reflect passive immunization rather than true alloimmunization, and thus, should not be misinterpreted as indicative of poor fetal outcomes. This distinction is critical for accurate clinical interpretation, effective patient counseling, and optimizing the cost-effectiveness and efficiency of antenatal care services.

In contrast, as anticipated, Group III exhibited a deterioration in prenatal fetal prognosis, characterized by elevated IDC titers (median titer: 1:256) and a higher rate of fetal involvement compared to the other groups. These findings align with literature, who reported a significant correlation between maternal anti-D antibody titers and the risk of fetal anemia, with higher antibody concentrations associated with increased red blood cell destruction and worsened fetal outcomes (30, 31).

In our study, the threshold IDC titer value for predicting documented neonatal anemia (Hb<14) was identified as 1:64, whereas the threshold associated with sonographic predictive evidence of fetal anemia was found to be higher, at 1:128. It can be logically stated that, every anemic fetus may not show sonographic detectable changes until a certain severity of the condition. Therefore, positive MCA-PSV changes with higher antibody titers may be interpreted as an expected finding. However, in either case, both thresholds (1:64 and 1:128) seem to be higher than the commonly accepted one (1:16) for Rh-alloimmunization (31). This brings to mind the idea of the need for updating commonly accepted thresholds to higher titers via newer and better powered studies.

One of the primary strengths of our study is its comprehensive and stratified evaluation of IDC test positivity in a large cohort of pregnant women, which allowed for detailed categorization of causes - distinguishing between true Rh D sensitization, prophylaxis-related positivity, and other non-anti-D-related etiologies. It suggests that prognosis can be determined by meticulous history taking and careful evaluation of underlying probable etiologies for positivity, independent of antibody titer levels. In this regard, in low resource settings where IDC titration is not feasible, it may be proposed that assessing the etiology of IDC positivity could still provide powerful insight into expected clinical outcomes and thus guide the health care provider. Unlike previous studies primarily centered on prevalence or neonatal outcomes, our study uniquely positions IDC positivity as both a diagnostic and prognostic marker, integrating maternal history, antibody titers, and alternative causes such as autoimmune disease and infections. This comprehensive approach offers a

more nuanced and clinically applicable interpretation of IDC test results in prenatal care.

Conclusion

This study underscores the importance of identifying underlying causes of IDC positivity to enable appropriate monitoring and timely interventions, thereby supporting the effective management of Rh disease through targeted screening strategies. IDC positivity - whether resulting from Anti-D immunoglobulin administration or other etiologies - appears to be associated with comparable antenatal and postnatal outcomes. It is imperative to routinely inquire about prior Anti-D immunoglobulin prophylaxis, particularly in multiparous women, and to perform an IDC test prior to prophylactic administration.

This is especially crucial in cases with elevated titers, as immunoglobulin-induced IDC positivity may interfere with an underlying Rh incompatibility, potentially affecting diagnosis and management. The presented data, although limited, may also bring the commonly accepted IDC titer thresholds up for discussion if supported by better powered studies. Multicenter, prospective studies with robust sample sizes are needed to validate and expand upon our findings and to optimize antenatal screening and counseling practices.

Declarations

Ethics approval and consent to participate declarations

All procedures conducted in this study adhered to the ethical standards of the institutional ethics committee (approval number: 13/09/2019/1977) and conformed to the principles of the 1964 Declaration of Helsinki and its subsequent amendments or comparable ethical guidelines.

IRB committee

Health Sciences University, Istanbul Training and Research Hospital, Department of Obstetrics and GynecologyDocument

No: E-96317027-514.10-225532551 Subject: 2011-KAEK-50

Date: 13/09/2019 (the document can be presented seperately if requested)

Consent to participate

This is a retrospective study, consent to participate is not applicable, and not required by our institution's IRB.

Availability of data and materials

Data is available and can be shared by editors and reviewers if necessary.

Declaration of competing interest

The authors report no declarations of interest.

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Concept- K. O. C and D.C.K. Design and data collection- K. O. C Analysis/Interpretation- G. A and D.C.K ; Literature search- K. O. C; Writing- K. O. C., Critical Reviews- D.C.K .and F.F.V.

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Consent for publication

Not applicable.

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